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Effect of cyclic AMP on acidification in the isolated turtle bladder

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Effect of cyclic AMP on acidification in the isolated turtle bladder. Cyclic AMP (10 mM) has been demonstrated to inhibit hydrogen ion secretion in the isolated turtle bladder. These experiments were designed to study the effect of cyclic AMP on hydrogen ion secretion in the isolated turtle bladder using both the pH stat and the reverse short circuit current techniques. Sodium transport was measured as the short circuit current. Studies were carried out at 0% CO_2 and 1% CO_2 at pH 7.4. Cyclic AMP (10 mM) was added to either the serosal or mucosal solutions, and hydrogen ion secretion was measured from 0 to 120 min. In the presence or absence of carbon dioxide, cyclic AMP and dibutyryl cyclic AMP had no effect on hydrogen ion secretion in fasted turtles. The addition of theophylline (10 mM) to the serosal solution, with or without cyclic AMP had no effect on proton secretion. Sodium transport was unchanged from control following serosal or mucosal addition of 10 mM cyclic AMP in the presence or absence of carbon dioxide. In chronically bicarbonate-loaded turtles proton secretion was the same as control fasted turtles. In these animals, however, serosal administration of 10 mM cyclic AMP significantly stimulated bicarbonate secretion. Stimulation of bicarbonate secretion occurred in the presence of a 20 mM bicarbonate gradient. When there was no bicarbonate gradient, cyclic AMP was without effect; cyclic AMP had no effect on bicarbonate permeability when measured in the presence of acetazolamide. These results indicate that cyclic AMP has no effect on hydrogen ion secretion or sodium transport in the isolated turtle bladder when studied at two different rates of acidification (0 and 1% CO_2). Cyclic AMP appears to stimulate active bicarbonate secretion.

Effet de l'AMP cyclique sur l'acidification dans la vessie de tortue isolée. Il a été montré que l'AMP cyclique (10 mM) inhibe la sécrétion d'ion hydrogène dans la vessie de tortue isolée. Ces expériences ont été conçues pour étudier l'effet de l'AMP cyclique sur la sécrétion d'ion hydrogène dans la vessie de tortue isolée, en utilisant des techniques de pH stat et de courant de court-circuit inversé. Le transport de sodium a été mesuré comme courant de court-circuit. Les études ont été faites à 0% CO_2 et à 1% CO_2 à pH 7,4. L'AMP cyclique (10 mM) était ajouté aux solutions séreuses ou muqueuses, et la sécrétion d'ions hydrogène était mesurée de 0 à 120 min. En présence ou en l'absence de carbone díoxyde, l'AMP cyclique et le dibutyryl AMP cyclique n'avaient pas d'effet sur la sécrétion d'ions hydrogène chez les tortues à jeun. L'addition de théophylline (10 mM) à la solution séreuse, avec ou sans AMP cyclique, n'avait pas d'effet sur la sécrétion de protons. Le transport du sodium n'était pas différent du contrôle après addition séreuse ou muqueuse de 10 mM d'AMP cyclique en présence ou en l'absence de carbone díoxyde. Chez des tortues en surcharge chronique en bicarbonates, la sécrétion de protons était la même que chez des tortues contrôles à jeun. Cependant, chez ces animaux l'administration séreuse de 10 mM d'AMP cyclique stimulait significativement la sécrétion de bicarbonates. La stimulation de la sécrétion de bicarbonate se produisait en présence d'un gradient de bicarbonate de 20 mM. Quand il n'y avait pas de gradient de bicarbonates, l'AMP cyclique était sans effet; l'AMP cyclique n'avait pas d'effet sur la perméabilité aux bicarbonates mesurée en présence d'acétazolamide. Ces résultats indiquent que l'AMP cyclique n'a pas d'effet sur la sécrétion d'ions hydrogène ou sur le transport du sodium dans la vessie de tortue isolée, étudiés à deux vitesses d'acidification différentes (0% et 1% de CO_2). L'AMP cyclique paraît stimuler la sécrétion active de bicarbonates.

Cyclic AMP is the second messenger mediating the response of many tissues to hormonal action [1–6]. The role of cyclic AMP in the turtle bladder is less well understood. Brodsky et al [7] reported a norepinephrine sensitive adenyl cyclase and cyclic AMP-dependent kinase from apical membranes of turtle bladder epithelium. The mucosal addition of agents known to increase cytosolic cyclic AMP (norepinephrine, histamine, and cholera toxin) stimulate acidification in the isolated turtle bladder [8–11].

A recent study by Lief, Mutz, and Bank [12] demonstrates that the serosal addition of cyclic AMP to the turtle bladder decreased proton transport to approximately 75% of control in 30 min. The serosal addition of theophylline, a phosphodiesterase inhibitor which allows higher levels of cyclic AMP to accumulate within the cell, caused a further decline in proton secretion. The combination of cyclic AMP and theophylline caused hydrogen ion secretion to decline to approximately 50% of control levels.

Several investigators have reported that cyclic AMP or phosphodiesterase inhibition stimulates active bicarbonate secretion in the turtle bladder [13, 14]. These studies however, were performed in the presence of zero sodium or chloride or both. The present studies were undertaken in an effort to define the mechanism by which cyclic AMP affects acidification in the presence of sodium and chloride in the isolated turtle bladder.

Methods

Turtles (*Pseudemys scripta*) were obtained from Lemberger farms (Oshkosh, Wisconsin, USA) and were fasted for 2 to 6 days. The bladder was excised and washed in Ringer's solution. The bladder was then cut into two hemibladders with one hemibladder serving as a paired control throughout the duration of the experiment. The bladders were mounted in a chamber (Lucite®, diameter, 8 cm^2), as previously described [15, 16], and were bathed with 10 ml Ringer's solution containing one of the

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two following compositions (mM): NaCl, 114.4; KCl, 3.5; $MgCl \cdot 6H_2$, 0.5; $CaCl \cdot 2H_2O$, 1.8 dextrose 5; Tris, 2.0. For these experiments the serosal solution was bubbled with 1% CO_2, and the mucosal solution was bubbled with compressed air. The pH of the serosa and mucosa was maintained at 7.4. The second solution contained (mM): NaCl, 114.4; KCl, 3.5; $MgCl \cdot 6H_2O$, 0.5; $CaCl \cdot 2H_2O$, 1.8; Na_2HPO_4, 2.0; dextrose 5. For these experiments both the serosal and mucosal solutions were bubbled with compressed air. The pH was maintained at 7.4. The compressed air was passed through a potassium hydroxide trap. The spontaneous potential difference (PD) was measured with 3 M potassium chloride-agar bridges and calomel half cells which were connected to a voltmeter (Model 600B Keithly Instruments, Inc., Cleveland, Ohio, USA). An automatic voltage clamp was used to supply current via the potassium chloride-agar bridges and silver chloride electrodes to nullify the spontaneous potential difference. Short circuit current (SCC) was measured by a Simpson microammeter. All experiments were performed in the short-circuited state. Bladders that failed to maintain a spontaneous PD greater than 15 mV during the first 60 min were discarded.

The rate of hydrogen ion secretion (RSCC) was measured as the current after sodium transport had been abolished by the serosal addition of 5×10^{-4} M ouabain. In some experiments, the rate of hydrogen ion secretion was measured using the pH stat technique. Continuous readings were made with a digital pH meter (Orion, Cambridge, Massachusetts, USA). Changes in the mucosal pH were titrated by an automated digital controller (Orion Model 872). A fall in the mucosal pH below 7.4 secondary to hydrogen ion secretion by the membrane, resulted in the activation of a servo-control mechanism delivering 0.01 N NaOH into the mucosal bath until pH returned to 7.4. Micromoles of hydrogen ion per hour per 8 cm^2 membrane area was calculated from the time interval and volume of sodium hydroxide delivered.

With others I have demonstrated that the reverse short circuit current (RSCC, that is, the current remaining following addition of ouabain) is identical to the rate of hydrogen ion secretion when measured with the pH stat method [15–17]. The RSCC was measured continuously during these experiments except for brief intervals when the PD was measured. In all the experiments in fasted turtles, the pH of the mucosal and serosal solution was maintained at 7.4.

After the SCC, RSCC or acidification was stable for at least 60 min, the pharmacologic agents were added to the mucosal or serosal side of one hemibladder. The other hemibladder served as control, and an equivalent amount of a diluent was added to its mucosal or serosal solution. The PD, SCC (or RSCC), and proton secretion were measured continuously for 120 min after the addition of 10 mM cyclic AMP, 1 mM dibutyryl cyclic AMP, and 10 mM theophylline. These parameters were measured for 30 min after the addition of 10^{-4} M acetazolamide (serosal).

A separate group of 16 turtles was soaked in 120 mM $NaHCO_3$ solution for 3 to 6 days and gavaged daily with 20 mmoles $NaHCO_3$ (alkali loaded turtles). Bladder urine was withdrawn for measurement of pH, P_{CO_2}, sodium, and potassium. Cardiac puncture was performed for measurement of pH and P_{CO_2}. The bladders were mounted as described above and acidification was measured using pH stat titration and RSCC techniques. In the experiments in which bicarbonate secretion was measured and the pH stat technique was employed using 0.01 N HCl as the titrant, the bladders were initially mounted in a symmetric solution containing (mM): NaCl, 94.4; KCl, 3.5; $MgCl_2$, 0.5; Na_2HPO_4, 2.0; $CaCl_2$, 1.8; Na_2SO_4, 10; dextrose, 10; pH 7.4, 226 mOsm/kg H_2O. After 2 to 3 hr stabilization 5×10^{-7} M ouabain was added, and when the H^+ current stabilized (RSCC), the mucosal pH was titrated with hydrochloric acid to a point at which the RSCC was zero (4.1 ± 0.02). The serosal fluid was then changed to one in which 20 mM $NaHCO_3$ replaced the solution containing 10 mM Na_2SO_4 and 10 mM dextrose (pH 8.0). The total rate of HCO_3 secretion into the mucosa was measured with pH stat titration using 0.01 N HCl as the mucosal titrant. Under these conditions a diffusional current of 0.33 μmoles/hr has been recorded by Cohen [18] due to the assymetry of the solutions. Cyclic AMP was added to the serosal solution after a stable rate of bicarbonate secretion was obtained and was followed for 45 min thereafter.

In a separate group of seven alkali-loaded animals the effect of cyclic AMP on bicarbonate secretion was measured in the presence of 10^{-4} M acetazolamide. These experiments, performed in the absence of active transport, were designed to assess the effect of the nucleoside on passive bicarbonate permeability.

To ensure the potency of cyclic AMP, water transport experiments were performed in isolated toad bladder as previously described [19]. Serosal addition of cyclic AMP (10 mM) stimulated water transport from 7.4 ± 1.6 to 139.6 ± 12.9 μl/cm^2/hr ($N = 8$, $P < 0.001$).

Table 1. Effects of serosal cyclic AMP and dibutyryl AMP on hydrogen secretion in the isolated turtle bladder perfused with and without carbon dioxide

		Baseline H^+ secretion *μAmps/8 cm²*	E/B H^+ secretion *% Control*
Group 1			
1% CO_2	Control ($N = 7$)	20.3 ± 4.7	95.7 ± 4.8
$P <$		NS	NS
1% CO_2	10 mM cyclic AMP ($N = 7$)	21.0 ± 4.6	99.0 ± 5.6
Group 2			
0% CO_2	Control ($N = 11$)	22.3 ± 2.5	105.1 ± 3.6
$P <$		NS	NS
0% CO_2	10 mM cyclic AMP ($N = 11$)	25.2 ± 1.5	100.8 ± 9.8
Group 3			
1% CO_2	Control ($N = 7$)	43.4 ± 7.7	98.9 ± 1.3
$P <$		NS	NS
1% CO_2	1 mM Dibutyryl AMP ($N = 7$)	49.3 ± 7.6	94.7 ± 2.4

Abbreviations: E, experimental; B, baseline.

Results

Effect of 10 mM cyclic AMP and 1 mM dibutyryl AMP on proton secretion

Table 1 shows the results of serosal addition of 10 mM cyclic AMP and 1 mM dibutyryl AMP on hydrogen ion secretion in the presence and absence of carbon dioxide. Of the eleven experi-

Table 2. Effect of 10 mM theophylline on hydrogen ion secretion by the isolated turtle bladder[a]

	N	E/B H+ secretion % Control	P	MPD
Control	14	98.4 ± 4.0		
			NS	−14.9 ± 12.6
Theophylline, *10 mM*	14	113.3 ± 9.0		
Cyclic AMP, *10 mM*	8	108.0 ± 9.2		
			NS	−5.1 ± 11.3
Cyclic AMP, *10 mM*, plus Theophylline, *10 mM*	8	113.3 ± 6.2		

Abbreviations: E, experimental; B, baseline; MPD, mean pair difference (control minus experimental) expressed as the percentage baseline of hydrogen ion secretion.

[a] All studies were performed in the absence of exogenous carbon dioxide and bicarbonate.

ments performed in the absence of carbon dioxide (that is, group 2) I measured acidification utilizing the pH-stat technique in five, and six used the RSCC technique. Because no effect was seen with cyclic AMP, the results were pooled, and the remainder of the experiments were performed with the reverse short circuit current. Baseline hydrogen ion secretion was comparable with each group of hemibladders, although there was some variation between groups (that is, group 3 acidified at a higher rate as compared to groups 1 and 2). At 60 min, there was no effect of the serosal addition of 10 mM cyclic AMP on hydrogen ion secretion, either in the presence of 1% CO_2 or in the absence of carbon dioxide and bicarbonate. Similarly, following the addition of 1 mM dibutyryl cyclic AMP, in the presence of 1% CO_2, no effect was seen on proton secretion. There was no difference in the PD between either the control or experimental groups (data not shown on Table 1).

In the absence of carbon dioxide, the mucosal addition of 10 mM cyclic AMP did not result in change in proton secretion (mean pair difference, control minus experimental, 17.3 ± 8.1%, $N = 8$, NS). In the presence of 1% CO_2 the mucosal addition of 10 mM cyclic AMP or 1 mM dibutyryl cyclic AMP had no effect on acidification (mean pair difference, control minus experimental, 14.1 ± 7.0%, $N = 7$, NS for the cyclic AMP group; −4.7 ± 3.1%, $N = 7$, NS for the dibutyryl cyclic AMP group).

Effect of 10 mM theophylline on hydrogen ion secretion

Table 2 shows that serosal addition of theophylline had no effect on hydrogen ion secretion by the isolated turtle bladder at 60 min in the absence of carbon dioxide and bicarbonate. In a separate series of experiments, the addition of theophylline to cyclic AMP treated bladders had no effect on proton secretion.

Effect of 10 mM cyclic AMP and 1 mM dibutyryl AMP on sodium transport (SCC)

Table 3 shows that the serosal addition of 10 mM cyclic AMP on SCC had no effect in the absence of carbon dioxide or bicarbonate. Similarly, 1 mM dibutyryl AMP (1% CO_2) had no effect on sodium transport (10 mM cyclic AMP was without effect on SCC, data not shown). The mucosal addition of 10 mM cyclic AMP in the absence of carbon dioxide did not alter sodium transport (mean pair difference, control minus experimental, 15.3 ± 9.9%, $N = 4$, NS).

Effect of acetazolamide on hydrogen ion secretion

Table 4 shows the effect of serosal addition of 10^{-4} M acetazolamide on hydrogen ion secretion in the absence of carbon dioxide. After 30 min hydrogen ion secretion was inhibited by 81.4 ± 8.1%, a value similar to that reported by Schwartz, Rosen, and Steinmetz [20]. When acetazolamide was added to the bladder pretreated with cyclic AMP, proton secretion was inhibited by 89.0 ± 5.0%, a value not significantly different from acetazolamide alone.

Effect of cyclic AMP on proton secretion and bicarbonate secretion from chronically alkali loaded turtles

I examined the effect of cyclic AMP on hydrogen ion secretion and bicarbonate secretion in animals which had been chronically alkali-loaded for 3 to 6 days. In 16 animals the arterial pH, P_{CO_2} and bicarbonate was 7.46 ± 0.03 and 54.8 ± 3.4 mm Hg and 35.9 ± 2.0 mEq/liter, respectively. Urine pH was 6.73 ± 0.12. Urine P_{CO_2} and bicarbonate was 43.0 ± 2.1 mm Hg and 2.6 ± 0.6 mEq/liter, respectively.

Proton secretion was measured using a pH stat technique and titration with 0.01 N NaOH in six bladders at pH 7.4 (mucosa and serosa) in the absence of bicarbonate. Table 5 shows that, in the presence of active transport, control hydrogen ion secretion was 2.62 ± 0.24 μmoles/8 cm^2/hr. Following 10 mM cyclic AMP proton secretion was not different from control (2.50 ± 0.18, $N = 6$, NS). When hydrogen ion secretion was measured using the RSCC technique, in the presence of ouabain, 10 mM cyclic AMP likewise had no effect. Sixty minutes after serosal cyclic AMP, RSCC was 110.9 ± 3.5% of control while that of the paired diluent treated hemibladder was 106.9 ± 4.1% of control ($N = 6$, NS).

Under conditions of active transport and in the presence of a 20 mM bicarbonate gradient (serosa), control bicarbonate secretion in alkalotic turtles was 2.15 ± 0.33 μmoles/8 cm^2/hr, ($N = 6$), a value similar to that reported by Cohen [18]. Serosal addition of 10 mM cyclic AMP caused a significant increase in bicarbonate secretion to 2.59 ± 0.28 μmoles/8 cm^2/hr as compared to control, ($P < 0.025$, Table 5). Thus, under conditions of active transport in the presence of a bicarbonate gradient, cyclic AMP significantly stimulates bicarbonate secretion.

Because bicarbonate secretion was enhanced in alkali loaded turtles by serosal cyclic AMP, I sought to determine whether or not this effect was due to an effect on passive bicarbonate permeability. To perform these experiments bicarbonate secretion was measured in the absence of active transport (that is, in the presence of acetazolamide).

In seven chronically alkali-loaded animals the arterial pH, P_{CO_2} and $pHCO_3$ was 7.57 ± 0.01, 77.71 ± 9.21 mm Hg, and 68.83 ± 6.14 mEq/liter, respectively. Urine pH was 7.10 ± 0.18 ($N = 7$). Urine P_{CO_2} and bicarbonate was 57.8 ± 2.9 mm Hg and 16.0 ± 7.47 mEq/liter, respectively ($N = 6$). As shown in Table 5, when serosal cyclic AMP was added to one hemibladder (the other serving as time control), there was no change in passive bicarbonate permeability (1.59 ± 0.06 versus

Table 3. Effect of serosal 10 mM cyclic AMP and 1 mM dibutyryl AMP on electrical parameters and sodium transport in the isolated turtle bladder

Conditions		N	Baseline SCC $\mu AMP/8\ cm^2$	E/B SCC % of control	Potential difference, mV		
					B	P	E
0% CO_2	Control	13	198.7 ± 28.6	95.6 ± 1.9	36.3 ± 3.4	NS	32.9 ± 3.4
				NS			
	10 mM cAMP	13	234.8 ± 41.8	89.8 ± 4.9	28.2 ± 4.5	NS	23.5 ± 4.0
1% CO_2	Control	4	179.0 ± 32.2	92.3 ± 2.0	36.8 ± 6.9	NS	33.0 ± 6.1
				NS			
	1 mM Dibutyryl AMP	4	160.0 ± 14.8	98.8 ± 7.1	40.8 ± 8.2	NS	40.5 ± 9.7

Abbreviations: SCC, short circuit current; E, experimental; B, baseline.

Table 4. Effect of 10^{-4} M acetazolamide on hydrogen ion secretion by the isolated turtle bladder

	N	H+ secretion $\mu Amps/8\ cm^2$	Potential difference mV
Control	6	23.4 ± 4.0	7.3 ± 2.1
$P <$		0.05	0.05
Acetazolamide, $10^{-4}M$[a]	6	5.2 ± 3.1	2.2 ± 1.2
Cyclic AMP	6	29.8 ± 2.7	10.0 ± 2.8
$P <$		0.05	0.05
Cyclic AMP + Acetazolamide[a]	6	3.1 ± 2.1	2.0 ± 1.8

[a] Measurements were made 30 min after serosal addition of acetazolamide.

Table 5. Effect of cyclic AMP on J_H and J_{HCO_3} in the presence and absence of active transport[a]

Condition	Control	P <	Cyclic AMP
	$\mu moles/8\ cm^2/hr$		
Control (+active transport)			
J_H ($N = 6$)	2.62 ± 0.24	NS	2.50 ± 0.18
J_{HCO_3} ($N = 6$)	2.15 ± 0.33	0.025	2.59 ± 0.28
Acetazolamide (−active transport), 10^{-4} M			
J_{HCO_3} ($N = 7$)	1.62 ± 0.10	NS	1.59 ± 0.59

Abbreviations: J_{HCO_3}, 20 mM $NaHCO_3$ gradient (serosa to mucosa); J_H, hydrogen ion secretion.

1.62 ± 0.10 μmoles/8 cm^2/hr in control and cyclic AMP, respectively $N = 7$, NS).

Discussion

These studies were undertaken to examine the mechanism whereby cyclic AMP affects acidification in the turtle bladder. The results show that exogenous cyclic AMP had no effect on proton secretion in the bladders of fasted turtles either during high (1% CO_2) or low (0% CO_2) proton secretory rates. Inhibition of phosphodiesterase activity also was without effect. In chronically alkali-loaded turtles, by contrast, cyclic AMP significantly stimulated bicarbonate secretion only in the presence of a bicarbonate gradient. When active hydrogen transport was inhibited with acetazolamide, cyclic AMP had no effect on bicarbonate secretion, indicating that cyclic AMP did not affect passive bicarbonate permeability.

Cyclic AMP is an important "second" messenger in many aspects of cell function [1–6]. In the turtle bladder, however, its importance is less well established. Lief, Mutz, and Bank [12] reported that serosal cyclic AMP inhibited proton secretion in the isolated turtle bladder suggesting an effect on the modulation of hydrogen secretion. In the absence of carbon dioxide (that is, low hydrogen ion transport rates) cyclic AMP decreased proton secretion to 75% of the control values [12]. When a phosphodiesterase inhibitor was added to the bathing medium, thereby presumably further elevating cytosolic cyclic AMP levels, there was an additional significant decrease in proton secretion. When cyclic AMP was added in the presence of carbonic anhydrase inhibition, cyclic AMP markedly decreased proton secretion; and if theophylline was added, proton secretion fell to zero. This study suggests that cyclic AMP is an important mediator of proton transport in the turtle bladder though direct measurement of an increase in cytosolic cyclic AMP was not documented.

I was unable to confirm these findings on proton secretion under conditions of low transport rates (0% CO_2) or high transport rates (1% CO_2) using cyclic AMP, dibutyryl AMP, or theophylline. These studies were performed using both the reverse short circuit current and the pH stat technique. While the reason for this discrepancy is not completely clear, a difference in experimental design is one explanation. I used the paired hemibladder techniques as the time control whereas theirs did not. Their study used bladders from different animals as controls. If the experimental bladders spontaneously decreased proton secretion with time while the controls did not, this would be interpreted as an effect of cyclic AMP when, in fact, no such change occurred.

Another possibility (although an unlikely one, see below) for the divergent results is that despite the absence of serosal carbon dioxide, the bladders used in the experiments of Lief, Mutz, and Bank [12] had intrinsically high bicarbonate secretory rates. If cyclic AMP stimulated bicarbonate secretion, titrametrically this would be equivalent to a decrease in proton secretion. This issue deserves further comment.

Two studies have presented evidence that an increase in bicarbonate secretion is the mechanism whereby cyclic AMP exerts its effect on membrane transport [13, 14]. Ehrenspeck

[13] studied the effect of cyclic AMP on acidification in turtle bladders bathed by chloride-free solutions. In some experiments, choline was substituted for sodium. Satake, Durham, and Brodsky [14] performed experiments in bladders bathed in solutions that did not contain sodium or chloride. In both experiments [13, 14] cyclic AMP and the inhibition of phosphodiesterase stimulated bicarbonate secretion. In the study by Satake, Durham, and Brodsky [14] the effect of cyclic AMP was not seen in acidotic turtles. The effect of cyclic AMP was most marked in alkali-loaded turtles, although it was seen in the postprandial state (12 to 14 hr fast) and postabsorptive state (7-day fast).

Because these studies were performed in states of marked ion substitution, I repeated the study in a normal sodium and chloride medium. Turtles were made chronically alkalotic by gavage and when the proton transport was studied at pH 7.4 in the absence of bicarbonate, the turtles continued to acidify the mucosal medium (RSSC lumen+). Serosal cyclic AMP under these conditions had no effect, which is the reason a high spontaneous rate of bicarbonate secretion is an unlikely explanation for the findings of Lief, Mutz, and Bank [12]. Chronic alkalosis (pH 7.54, P_{CO_2} 48 mm Hg, $pHCO_3$ 40 mEq/liter) has been shown to have no effect on the rate of proton secretion (57 ± 6 μAMPS) nor to affect the pH at which acidification stops (4.43 ± 0.06) [18]. When a bicarbonate gradient was present (20 mM $NaHCO_3$) on the serosa and bicarbonate secretion was studied in chronically alkalotic turtles, cyclic AMP significantly enhanced alkalinization of the lumen. Alkalosis has been reported previously to cause a 40% increase in the rate of bicarbonate secretion from 1.17 ± 0.14 to 1.63 ± 0.11 μmoles/8 cm^2/hr [18]. In this study, bicarbonate secretion averaged 2.1 μmoles/8 cm^2/hr during chronic alkalosis, and cyclic AMP further significantly increased bicarbonate secretion to 2.6 μmoles/8 cm^2/hr.

Bicarbonate secretion by the turtle bladder has been studied by several investigators [13, 14, 21–23]. While it is not completely clear whether this is an electrically neutral exchange (bicarbonate for chlorine) or electrogenic, it consists of at least two components. The first component of bicarbonate secretion is passive permeability. All experiments must be performed in the presence of a large bicarbonate gradient (20 mM HCO_3, serosa). The second component of bicarbonate secretion is active, and as such, is carbonic anhydrase-dependent. Under conditions of zero chloride (or zero sodium), Ehrenspeck [13] found evidence that cyclic AMP stimulates bicarbonate secretion by the latter mechanism; the effect of cyclic AMP (or phosphodiesterase inhibition) was not seen when the bladders were pretreated with carbonic anhydrase inhibitors. The present study, under conditions of normal sodium and chloride concentrations, shows that cyclic AMP stimulates bicarbonate secretion in alkalotic turtles.

I investigated the mechanism of this action by studying the effect of cyclic AMP in the presence of acetazolamide in the chronically alkali-loaded turtle. I reasoned that if cyclic AMP still stimulated bicarbonate secretion in the presence of carbonic anhydrase inhibition, then the likely mechanism of action was via an increase in passive bicarbonate permeability. On the other hand, if cyclic AMP had no effect on bicarbonate secretion under these conditions, then one could invoke an active mechanism as Ehrenspeck suggested [13]. Bladders from chronically alkalotic turtles were placed in a buffer containing 10^{-4} M acetazolamide. The rate of bicarbonate secretion fell approximately 50% of control (that is, 2.1 to 1.6 μmoles/8 cm^2/hr). While this value is less than that reported by others, it should be pointed out that this is a comparison between groups, and that the two groups of alkali-loaded animals are not totally comparable. The animals were studied at different times (summer and winter), and the winter group became more alkalotic when given the same experimental protocol. In addition, the bladders from the winter groups were excised and placed immediately into buffer containing acetazolamide. Despite these differences, cyclic AMP had no effect on bicarbonate secretion in the presence of carbonic anhydrase inhibition. Because these studies were performed in the presence of a large serosal bicarbonate gradient they show that cyclic AMP does not increase the passive component of bicarbonate permeability. Thus, the mechanism for the effect of cyclic AMP on bicarbonate secretion in the alkalotic turtle appears to be mediated by active transport, occurring in parallel with active proton secretion. Ehrenspeck [24] recently presented further evidence in this membrane that cyclic AMP and phosphodiesterase inhibitors stimulate only bicarbonate secretion, while the calcium ionophore A23187 both stimulates bicarbonate secretion and inhibits concurrent proton secretion.

In conclusion, these studies show that cyclic AMP does not inhibit proton secretion or sodium transport. Cyclic AMP stimulates bicarbonate secretion in chronically alkalotic turtle bladders in the presence of sodium and chloride, only in the presence of a large bicarbonate gradient. This effect appears to be an active process in that pretreatment with the carbonic anhydrase inhibitor, acetazolamide, abolishes the effect. These results suggest that bicarbonate secretion and proton secretion occur in parallel in this membrane and that cyclic AMP affects only the former process.

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